Evaluating of Serum Adenosine Deaminase Isoenzymes in Lung Contusion

Akciğer Kontüzyonunda Serum Adenozin Deaminaz İzoenzimlerinin Değerlendirilmesi

Akciğer Kontüzyonunda ADA İzoenzimleri / ADA Isoenzymes in Lung Contusion

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Özet

Amaç: Akciğer kontüzyonlu ratların serumlarında adenozin deaminaz (ADA) izoenzimlerinin aktivitelerini araştırmayı amaçladık. Gereç ve Yöntem: On iki tane erkek wistar albino sıçanda toraks üzerine hareketli platform ile 50 cm yükseklikten bir silindirik ağırlık bırakılarak akciğer kontüzyonu oluşturuldu. Travmadan 24 saat (n=6) ve 72 saat (n=6) sonra sıçanlar öldürüldü. Kontrol (n=6) (travma uygulanmayan) ve travmatik sıçanların serumlarında ADA izoenzimleri ölçüldü. Bulgular: Bizim sonuçlarımız kontüzyon sonrası 72 saatte serum total ADA aktivitelerinde önemli derecede azalma olduğunu göstermiştir. Kontüzyon sonrası 24 ve 72 saatteki ADA1 aktivitesinde kontrolle karşılaştırıldığında önemli bir düşüş vardır. Diğer taraftan, 24 ve 72 saatte ADA2 aktivitesindeki artış istatistiksel olarak anlamlı değildir. Sonuç: Sonuç olarak, serum ADA2 izoenzimi akciğer kontüzyonunda inflamatuvar yanıt nedeniyle baskın izoenzim haline gelmiştir. Ancak serum ADA1 aktivitesini etkileyen düzenleyici mekanizmaları açıklamak için ileri çalışmalara ihtiyaç duyulmaktadır.

Anahtar Kelimeler

Adenozin Deaminaz; Akciğer Kontüzyonu

Abstract

Aim: We aimed to investigate the activity of ADA isoenzymes in serum of rats with lung contusion Material and Method: Lung contusion was induced in twelve male wistar albino rats by dropping a cylindrical weight from a height of 50 cm with a mobile platform positioned over the thorax. Rats were killed at 24 hour (n=6) and 72 hour (n=6) after contusion. ADA isoenzymes were measured in serum traumatic and control (n=6) (uninjured) rats. Results: Our results indicated that serum total ADA activities were significantly decreased at 72 h after contusion. There was a significant decreased in ADA1 activity at 24 and 72 h after contusion when compared with controls. On the other hand, the increase in the ADA2 activity at 24 h and 72 h was not statistically significant. Discussion: In conclusion, serum ADA2 became predominant isozyme because of the inflammatory response in the lung contusion. However, further studies are needed to elucidate the regulatory mechanisms that effect the activity of serum ADA1.

Keywords

Adenosine Deaminase; Lung Contusion

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Introduction

Lung contusion is a significant problem in blunt chest trauma. Lung contusion injury revealed inflammatory response in the lung tissue. There were increased numbers of inflammatory cells within the alveoli and interstitium at 24 h after contusion due to inflammation [1,2]. It was demonstrated that lung contusion induces several systemic effects through the inflammatory mediators [2].

Adenosine deaminase (ADA) is an enzyme of the purine metabolism which degraded adenosine to hypoxanthine and inosine [3]. ADA plays essential role for the differentiation and proliferation of lymphocytes and the monocyte–macrophage cell system [4]. It has been two iso-enzymes form, adenosine deaminase 1 (ADA1) and adenosine deaminase 2 (ADA2) [5]. There are several reports that serum ADA1 and ADA2 activities has been increased or decreased in several diseases where cellular immunity is stimulated [6,7].

In the literature, ADA isoenzymes in serum have not been studied in trauma. Our previous investigation found decreased activity of total ADA in lung contusion while examining the effects of ghrelin on lung contusion [8]. Therefore, we decided to examine ADA isoenzyme activity in serum of traumatic rats. The aim of this study was to investigate the activities of ADA1 and ADA2 in serum after lung contusion and to evaluate the relationship between these enzyme activities.

Material and Method

Eighteen male wistar albino rats weighing 300–400 g were purchased from the Experimental Research Centre of Zonguldak Bulent Ecevit University (formerly Karaelmas University) Faculty of Medicine. All rats were on a 12-h light, 12-h dark cycle at 20-21 ^{0}C and provided with food and water ad libitum. The protocols were approved by the Zonguldak Karaelmas University Animal Care and Use Committee. All rats were divided into uninjured rats (control, n=6) and two groups of traumatic rats: at 24 hour (n=6) and 72 hour (n=6) following trauma were decapitated. Traumatic rats were anesthetized with ketamin-xylazine (60/10 mg/kg, im) and was performed lung contusion. Lung contusion model involves dropping a cylindrical weight (400 g) from a height of 50 cm onto a mobile lexon platform positioned over the thorax trauma [9]. At 24 h and 72 h after trauma, all animals were sacrificed. After decapitation, blood was collected by cardiac puncture, and the plasma was stored at 80 ^{0}C for the determination of ADA1 and ADA2 activities.

ADA activity (U/L) was assayed with a commercial kit (BEN, Milano, Italy) that is based on the enzymatic colorimetric method using Shimadzu UV 1601 spectrophotometer (Shimadzu Corporation, Kyoto, Japan). Adenosine is deaminated by ADA and the ammonia obtained from this reaction reacts with α-ketoglutarate. The latter reaction is catalysed by glutamate dehydrogenase. The enzyme activity was proportional to the decrease in absorbance of NADH and was measured at 340 nm. The detection limit of the assay was 3–150 IU/L with intra- and inter-assay coefficients of variable less than 5%. ADA2 activity was measured using the same technique in the presence of a potent selective inhibitor of ADA1, erytro-9-(2-hydroxy-3-nonyl) adenine (EHNA) (Sigma-Aldrich). The ADA1 activity is then calculated by subtracting the ADA2 activity from the total ADA activity.

Statistical analyses

All data were analyzed using SPSS 13 software and presented as means ± standard deviation. Differences between the groups were evaluated by Kruskal–Wallis one-way analysis of variance and Mann-Whitney U test. Differences in values were considered significant at $p < 0.05$.

Results

Total ADA and its isoenzymes levels in serum are shown in Table 1. Compared with controls, the concentrations of serum total ADA was significantly decreased at 72 h after contusion ($p < 0.05$). There was a significant decreased in ADA1 activity at 24 and 72 h after contusion when compared with controls ($p < 0.05$). No significant difference was found between post-contusion 24 h and 72 h in ADA1 isoenzyme levels. On the other hand, the increase in the ADA2 at 24 h and 72 h was not statistically significant (Figure 1).

Table 1. The activities of total ADA and its isoenzymes in serum control, post-contusion 24 h and 72 h.

	Control (n=6)	Trauma 24 h (n=6)	Trauma 72 h (n=6)
ADA (U/L)	32.6	28.9	22.9
ADA1 (U/L)	19.9	12.1	8.9
ADA2 (U/L)	12.4	16.8	14.1

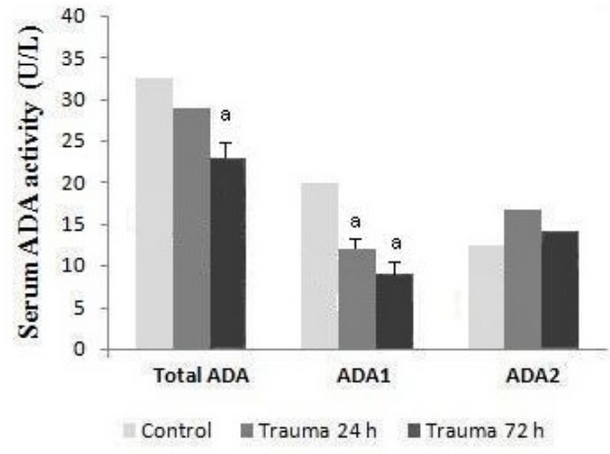

Figure 1. Comparison of control, 24 h and 72 h after post-contusion in serum of ADA isoenzymes
Data are mean ± SD, $^a p<0.05$ versus control group

Discussion

During acute tissue injury, extracellular nucleotide levels are significantly increased due to the release of ATP or ADP from intracellular to extracellular space. Extracellular nucleotides are rapidly hydrolyzed to adenosine [10,11]. ADA degrades either adenosine or 2-deoxyadenosine to inosine or 2-deoxyadenosine, respectively. Also, ADA plays an important role in the proliferation and differentiation of lymphocytes, blood monocytes and macrophages [3].

The ADA1 isoenzyme is found in all cells, with the highest activity in lymphocytes and monocytes. The primary role of ADA1 is to eliminate derivatives of adenosine and deoxyadenosine [12]. We can postulate that serum ADA1 activity is decreased in trau-

matic rats, which favors increased adenosine levels. Adenosine is a regulatory nucleoside that is generated in response to cellular stress and damage [13]. A recent study demonstrated that adenosine receptor signaling was an important protective role for during in lung protection from acute lung injury [14].

Macrophages are a significant source of serum ADA2 activity [15]. Raghavendran et al. [1] demonstrated that increased neutrophil accumulation in the lung parenchyma following increase in alveolar macrophage infiltration and monocytic response at 48 h after contusion. Conlon et al [15] demonstrated tissue macrophages activated during an inflammatory challenge may be a more abundant source of ADA2 than are circulating monocytes. They reported that ADA2 representing became the predominant isozyme in serum collected from septic rats, while ADA1 representing the predominant proportion of total ADA activity in nonseptic rats. ADA2 normally has a much lower capacity to catalyze adenosine deamination than ADA1 [16]. ADA2 enzyme activity becomes optimal at high levels of adenosine, low pH and inflammation conditions [17,18], that can associated with lung cuntusion injury. We found that ADA2 became predominant isoenzyme in serum, which may be due to active tissue macrophages during lung contusion.

In conclusion, serum total ADA activity is significantly decreased, which is mainly due to decreased ADA1 isoenzyme. Further investigation should be performed to elucidate regulatory mechanisms that effect the activity of serum ADA1.

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